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A longer confinement period favors European wild rabbit (*Oryctolagus cuniculus*) survival during soft releases in low-cover habitats

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Abstract Rabbit restocking is widely used in Spain for conservation and/or hunting purposes; however, the success of rabbit restocking is generally low. Thus, many studies have assessed ways to reduce this problem, one of which is the use of a “soft-release” procedure whereby rabbits are acclimated to their release site for a variable time period prior to release. This study assessed the short-term effects of two soft-release confinement periods on the survival of rabbits during an experimental restocking program carried out in southwest Spain. The post-release survival rate of rabbits confined at the release site for six nights was significantly higher than that of rabbits confined for three nights. The longer acclimation period after rabbit translocation minimized mortality while rabbits adapted to their new environment.

Keywords Confinement period · Rabbit conservation · Radio tracking · Restocking · Soft release · Translocation

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Introduction

The wild rabbit (*Oryctolagus cuniculus*) is a keystone species in its original distribution, being the staple prey of more than 30 predators (Delibes-Mateos et al. 2008a) and is one of the most important small-game species in Spain (Angulo and Villafuerte 2004). However, rabbit populations have declined dramatically in the Iberian Peninsula over the last 50 years, mainly due to the viral diseases myxomatosis and rabbit hemorrhagic disease (Moreno et al. 2007). In addition, human-induced habitat changes (i.e., intensification of agriculture and habitat fragmentation) have accelerated the decline or local extinction of rabbits in many regions during the last century (Moreno and Villafuerte 1995).

As a result of the decline in rabbit populations, a variety of management measures have been implemented in recent decades to enhance rabbit recovery. Among these, rabbit restocking has been increasingly used, particularly in central–southern Spain, and from 1993 to 2002, thousands of rabbits were restocked in almost half of the hunting estates (Delibes-Mateos et al. 2008b). However, both scientific studies and managers' experience show that the success of restocking is generally low (Calvete et al. 1997; Letty et al. 2002). High rabbit mortality during the 10 days immediately following release appears to be the main limiting factor in rabbit restocking (Calvete et al. 1997). High initial mortality limits restocking success because it reduces the breeding stock and, consequently, the viability of the population (Letty et al. 2008). This high mortality can be related to factors such as stress and social factors. Among the stages involved in translocation programs, capture, captivity, transportation, and release are the most stressful (Teixeira et al. 2007). Many studies have assessed ways to reduce this problem, one of which is the use of “soft release,” whereby rabbits are acclimated to their new

environment in mammal holding pens (Jefferies et al. 1986; Short et al. 1992). Comparison of “soft” and “hard” (without an acclimation period) release methods have generally demonstrated improved survival and behavior benefits with soft releases (Bright and Morris 1994).

Different acclimation periods for wild rabbits have been applied in both natural (Calvete and Estrada 2004) and artificial warrens (Letty et al. 2000). However, no study has been carried out to determine the best acclimation period and its efficacy in increasing rabbit survival. We hypothesized that a longer confinement period would favor rabbit acclimation to the release site, decreasing the novelty environmental effect. Therefore, this study assessed the effect of two different confinement periods on the short-term survival of rabbits translocated to artificial warrens as part of a soft-release process.

Materials and methods

Study area

The experiment was conducted in one of four restocking plots in the compensatory ecological area of Los Melonares (south of the Sierra Norte Natural Park of Seville, SW Spain; Fig. 1a). This region has two main biotopes: Mediterranean grassland (70%) and scrubland (30%). Rabbit abundance was relatively low before restocking, but both mammalian and raptor predators were present (Rouco et al. 2008).

The translocation site consisted of a grassland field approximately 4 ha in size, where artificial rabbit warrens were built (Fig. 1b; Rouco et al. 2008). Water and commercial pellet food suppliers were situated close to each warren and available ad libitum. Each artificial warren was surrounded by a wire net fence (warren pen), embedded 50 cm into the ground and extending 100 cm above ground; each pen had three to five rabbit doors (Fig. 1c). The primary function of the warren pens was to reduce immediate dispersal of rabbits while the pen doors were closed and to facilitate acclimation. The confinement period was defined as the time that the warren pen remained closed. Food and water were supplied ad libitum inside each warren pen during the whole confinement period, being administrated daily at daylight to avoid unnecessary disturbance of rabbits and terrestrial predators (mainly nocturnal).

Experimental design

To assess the effect of different confinement periods on the survival of translocated rabbits, we randomly selected 38 of the 181 rabbits introduced to the translocation site for monitoring following release. Each of these rabbits was

fitted with a radio collar (approximately 25 g; BIOTRACK, Wareham UK). The 38 radio-collared (“tagged”) animals were distributed in two groups for release after a confinement period with two different durations (“release treatment groups”). For one treatment group, 15 (6 males, 9 females) rabbits were confined in the warren pen for three consecutive nights, and the pen doors were opened on the fourth day. For the other group, 23 (10 males, 13 females) rabbits were confined for six nights, and the pen doors were opened on the seventh day. Both tagged and untagged rabbits were released inside the artificial warrens. Thus, 2–5 tagged rabbits were released in each of 15 randomly selected warrens. The average number (\pm SE) of rabbits (tagged and untagged) per warren in the plot was 10.05 ± 1.74 . All rabbits were released to the warren pens within 24 h of being captured on a hunting estate approximately 300 km from Los Melonares. None of the released animals were vaccinated against viral diseases (myxomatosis and rabbit hemorrhagic disease).

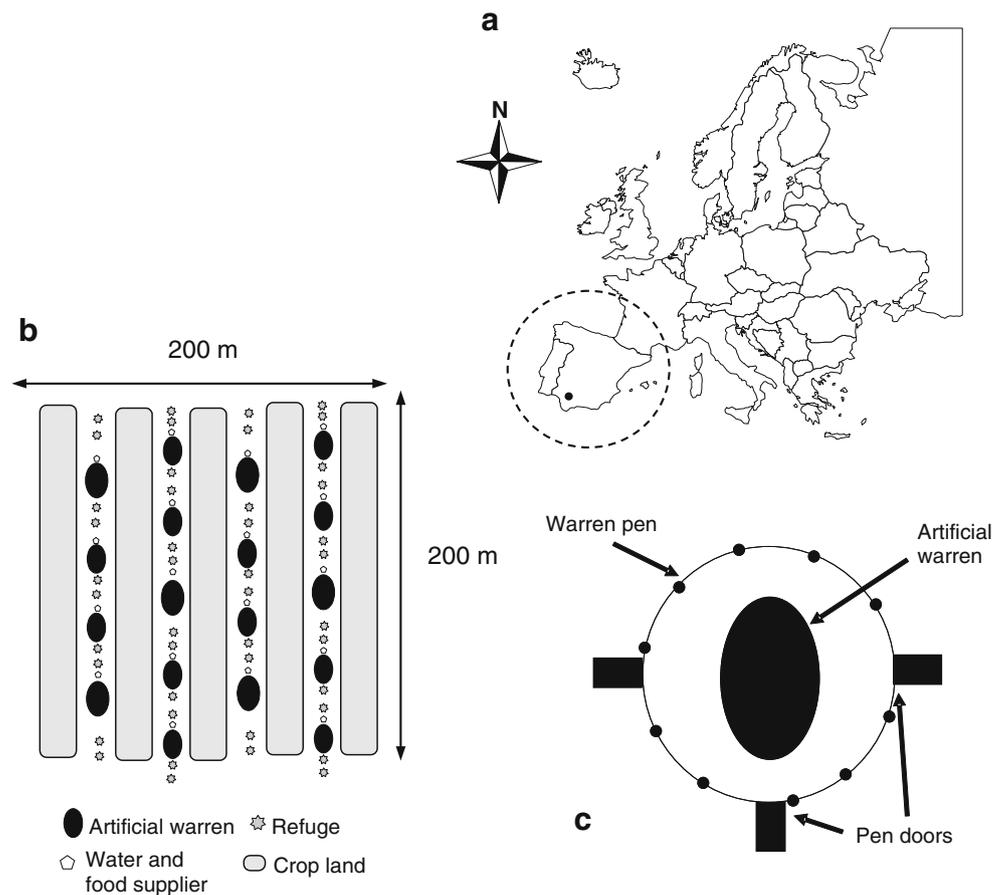
Survival of rabbits

All tagged rabbits were tracked daily during the confinement period and in the 10 days following the opening of the warren pen doors. Tracking to determine their position and whether they were dead or alive was done in daylight. Causes of death were determined by examining rabbit carcasses, identifying bite marks on the body and radio collar, examining the location of the remains of rabbits, and other signs. Predation was assigned to terrestrial carnivores when incisor marks on collars could be identified or when scats, rabbit cecum, or buried and half-buried corpses were found. On the other hand, rabbits assigned to predation by terrestrial carnivores could also be scavenged. Predation was assigned to raptors when evidence including feathers, characteristic tufts of torn-out fur, or remains of long bones were found. Deaths included in the “other causes” category included those assigned to disease and causes related to handling stress or aggression associated with social interactions (Calvete and Estrada 2004; Moreno et al. 2004). Deaths inside warrens were also included in this category because it was impossible to recover the corpses. Animals found dead on the n th day after release were considered to have survived $n-1$ days.

Data analysis

Survival and mortality rates, 95% confidence limits (CL) were calculated and compared using the Z statistic using MICROMORT and following the recommendations described by Heisey and Fuller (1985). Two-tailed Z test was used to test differences in survival between the two treatment groups as a function of the confinement period.

Fig. 1 **a** Location of the Los Melonares area (black dot) on the Iberian Peninsula. **b** Structure of a translocation plot comprising artificial warrens, refuges, and water and food suppliers. **c** Detail of an artificial warren (filled oval), location of the warren pen (dashed and dotted circle outline) and doors (filled rectangles)



Because of the different confinement period lengths, we compared daily survival rates between the two treatment groups during this first period (confinement period). To check for differences in survival between the two treatment groups during the critical period (following 10 days after confinement period), cumulative survival rates 10 days after the confinement period were compared.

Results

Most of the tagged animals survived the confinement period. However, one tagged animal in the three-night confinement period treatment pen died during the confinement period. This animal, found with the radio collar in its mouth, was not included in our analyses ($n=37$ tagged animals).

Of the 14 tagged rabbits that were confined for three nights inside the warren pen, two died during the confinement period; one was predated by a red fox (*Vulpes vulpes*) and the other one had no signs of predation (assigned to handling stress). In the six-night treatment, three animals were found dead during the confinement period. Two were found inside the warren, and another was

predated by a red fox. Daily survival rates were high for both confinement periods (0.86, 95% CL=0.694–1, for the shorter confinement period; 0.89, 95% CL=0.772–1, for the longer period) but not significantly different ($Z=0.42$; $P=0.676$). Survival rates of females and males during the confinement period did not differ significantly between the two treatment groups (three nights: $Z=0.23$, $P=0.180$; six nights: $Z=0.28$, $P=0.222$).

The cumulative survival rate for the 10 days following the opening of the warren pen doors was estimated for each treatment. Rabbits that were confined for six nights had significantly higher survival rates than those confined for three nights ($Z=2.06$, $P=0.039$; Fig. 2).

Most deaths occurred during the days immediately following the opening of the warren pen doors and mainly in the group with the shorter confinement period. Deaths in the group of animals from the longer confinement period treatment occurred throughout the following 10 days (Fig. 2). Predation by terrestrial carnivores was the main cause of death during the study. After release, rabbits that were maintained for the shorter confinement period were more frequently predated (mortality rate due to terrestrial predators=0.38, CL 0.09–0.67) than animals enclosed for the

longer period ($M=0.09$, CL 0–0.20), although the differences were only marginally significant ($Z=1.83$, $P=0.066$).

No differences were found between survival rates of females and males during the 10 days following release after the confinement period (three nights: $Z=0.67$, $P=0.501$; six nights: $Z=0.85$; $P=0.393$).

Discussion

The current assessment of the short-term effects of two soft-release confinement periods on the survival of rabbits during restocking found that the post-release survival rate of rabbits confined at the release site for six nights was significantly higher than that of rabbits confined for three nights. In addition, in terms of mortality during confinement, the present study found similar mortality rates regardless of whether rabbits were confined for three or six nights. In fact, contrary to what might be expected, we observed a slightly higher mortality rate during the shorter confinement period (12.9%), although no statistically significant differences were found. Therefore, at the end of the confinement period, an “underlying mortality” is acting, which seems to be due to handling related to the translocation process (i.e., capture, transport) and captivity itself (i.e., agonistic behavior; Teixeira et al. 2007), since most of the deaths occurred inside the warren or with no signs of predation or disease, during the confinement period.

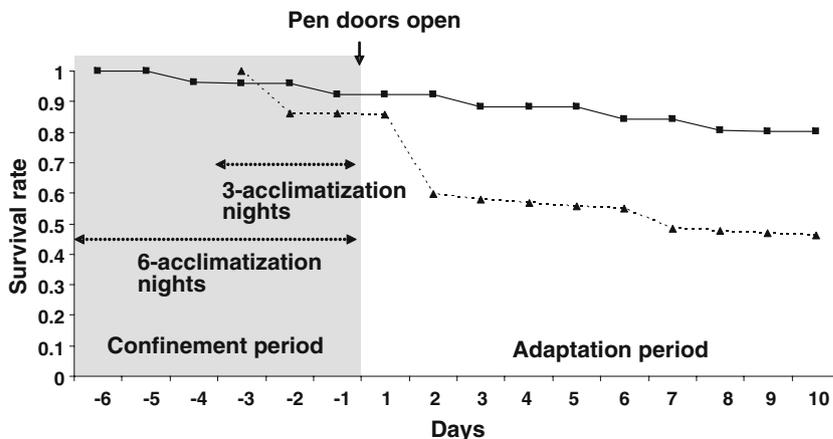
After the pen doors were opened and rabbits were allowed to move freely in the study area, most deaths were due to terrestrial predators (mainly foxes). This is in accordance with most previous studies on rabbit translocation (e.g., Calvete et al. 1997; Moreno et al. 2004; Letty et al. 2008). However, in our case, a lower mortality occurred among animals held for the longer confinement period. Although the reason for the lower mortality of rabbits confined for six nights is not known, it is likely that a three-day acclimation period is

insufficient for adequate settlement. We found that the higher mortality of the rabbits confined for three days seemed to be related to a higher proportion of these rabbits quickly leaving the warren after pen door opening, in many cases, on the day of pen door opening. This tendency to abandon the warren may indicate that three nights of confinement was not enough for all rabbits to feel safe inside the warren. Another explanation is that the rabbits that left the warren were subordinate animals. However, animals with six acclimation nights adapted better to their release warren. Moreover, in agreement with our hypothesis, 3 months later, most of the survivors (68%) remained in the warren where they were released (Rouco et al. 2008).

It is difficult to assess the optimal confinement period by comparing data from only two different time periods. However, if the relationship between nights confined and associated mortality were linear, it would be possible to estimate such regression with the mortalities obtained at the end of each period (0.54 and 0.20 for three- and six-night confinement periods) and the mortality obtained in a previous study conducted with no acclimation period (zero nights of acclimation, 0.97; Calvete et al. 1997). According to this analysis, mortality becomes similar to the previously mentioned underlying mortality due to confinement when rabbits are confined for 6.41 days ($R^2=0.995$). Although the relationship between nights confined and mortality is likely nonlinear, comparison of the mortality during the adaptation period for the three- and six-night rabbits indicates that six nights is close to the optimal period, while three days is far from optimal. Further studies assessing other confinement periods would help to better define the optimal time rabbits should remain confined in the pen.

In assessing our results, some additional considerations should be made. On the one hand, it is possible that other factors could also affect the optimum period of confinement. Our study was carried out in a low-cover habitat, building artificial warrens which were basically the main

Fig. 2 Cumulative survival rates per day for each treatment group (three-nights of acclimation, and six-nights of acclimation) during the confinement period plus the following 10 days that compound the adaptation period



refuge for rabbits, and it has been previously shown that cover may alter the dispersal distance (and therefore survival) of the released rabbits (Calvete and Estrada 2004). Finally, and more importantly, Letty et al. (2008) observed that relevant differences in survival of translocated rabbits could depend more on the quality of the habitat in the release area than on the length of the acclimation period. Therefore, gamekeepers and conservationists should take into account not only the suitability of the habitat but also acclimation when releasing rabbits in translocation programs.

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